UNIVERSITY OF GONDAR

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE

DEPARTEMENT OF BIOLOGY

APPLIED MICROBIOLOGY POSTGRADUAT PROGRAM

Evaluation of Microbial Induced Deterioration and Use of Botanicals as a Means of Management option on Historical Heritages of Gondar

A thesis submitted to the department of biology in partial fulfillment for the requirements degree of Master of Science in Applied Microbiology

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JUNE, 2015

GONDAR, ETHIOPIA

AUTHOR'S DECLARATION

The research work in this thesis entitled "EVALUATION OF MICROBIAL INDUCED DETERIORATION AND USE OF BOTANICALS AS A MEANS OF MANAGEMENT OPTION ON HISTORICAL HERITAGES OF GONDAR" was carried out by me under the supervision of Dr. Samuel Sahile in the Department of Biology, University of Gondar, for the award of M.Sc. Degree in Applied Microbiology.

I declare that this work is original and has not submitted to any other University or institute.

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Acknowledgment

All things gone as He order which was the best way to success my destination and I forward thank and I wish ever honor to almighty God.

I would like to express whole hearted thank to my advisor Dr. Samuel Sahile for his extreme positiveness, continuous follow up sentiment, and encouragement during my thesis. His auspices not only ideally but also materially, he taught me academically behaviorally and practically.

Staff of Department of Biology and microbiology laboratory assistants play big role by facilitating necessary conditions for the accomplishment of my practical work and I would like to thank them.

To my Dell, I know from whom you sent and you commit your mission. Your support was all-round and I climb the mountain because your love was the back force.

Last but not least I would like to thank my family for their financial support and encouragement.

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List of Acronyms

- PDA Potato Dextrose Agar
- UNESCO United Nation Educational Science and Cultural Organization
- WHC World Heritage Committee
- WHO World Health Organization
- ANOVA Analysis Of Variance
- FDRE Federal Democratic Republic of Ethiopia

Abstract

Loss of historical heritages is not only due to aging and environmental factors but also damaged as a result of microbial induced deterioration. Microbial induced deterioration of historical heritages occurs due to the metabolic versatility of microorganisms supported by favorable climatic and environmental condition like moisture, pH, temperature and substrate. This study was aimed to evaluate microbial induced deterioration of historical heritages of Gondar town and determine the efficacy of common medicinal plants as management option for the existing problem. Sixty swab samples were taken from deteriorated area of wall paintings, wood surface and wall/concrete of the three historic places of Gondar town namely Fasil castle (world heritage), Qwesquam, and Debre Berhan Selassie churchs. The swab samples were taken from deteriorated sites and cultured on right growth media and characterize based on the Bergey's Manual of Systematic Bacteriology. Preparation of crude extract of *Azadirachta indica* (Neem), *Datura Stramonium* (Atsefaris) and *Ricinus communis* (Gulo) were done using different solvent and screening of plant phytochemicals were done using indicator chemicals. A total of 7 bacteria and 11fungal species were identified from the deteriorated area of historic heritage. Among them *Staphylococcus epidermidis* and *Aspargilus niger* were dominant. Anti microbial effect of the selected plant extract were done, the acetone/chloroform extract of *Azadricheta indica* show highest inhibition zone for *S.epidermidis* while the acetone/chloroform extract of *Datura stramonium* show highest inhibition zone for *A.niger.* The acetone/chloroform extract of *A.indica* and *R.comminus* reveal MIC (25 mg/ml) for *S.epidermidis* and 25 mg/ml ethanol and acetone/chloroform extract of the three medicinal plant records as MIC for *A.niger*. The historical heritages of Gondar town subjected for microbial induced deterioration. The efficacy of botanicals against the selected isolates were different depend on the solvent used and the type of plant bioactive compound.

Key words/Phrases;- Historical heritage, Biodeterioration, paint, wood, Botanicals, Phytochemicals

Introduction

Historical heritages are architectural and aesthetical finger print of the pre-historic people, which is made from different organic and/or inorganic materials. Historic heritages reveal belief, living style, and civilization of per-historic peoples with magnificent architectural and aesthetical skill. They are very impressive and many people's around the world fascinated with them. Historical heritages provide income; Peoples invest money and visit them to get recreational value as well as to grasp information about history of the ancient peoples, this could result economic support for countries (www.tourismethiopia.gov.et/ Accessed on July 10, 2014).

Historical heritage damages due to different factors such as physical, chemical, and biological factors. Biological factors induce the involvement of organisms called Biodeterioration; biodeterioration refers to undesirable changes in a material, caused by living organisms (Allosopp, 2011). Predominantly fungi and bacteria cause serious aesthetical destruction of paintings, costumes, stone monuments, mummies, books and manuscripts; they inhabit and penetrate into the materials, resulting in material loss, due to acid corrosion, enzymatic degradation and mechanical attack of fungal hyphae. Living organisms form specific communities that interact in many different ways with mineral materials and their external environment. This complex phenomenon occurs in conjunction with many physical and chemical destructive processes (Ahinkafi and Haruna, 2013).

Microorganisms mainly bacteria and fungi are highly involved in deterioration of historical heritages. Due to their versatile metabolic activities, they do have wide variety of substrate to challenge and consume it. The component of historic material can be the source of food otherwise organic and inorganic pollutant serve as substrate (Gross *et al.,* 2006). Most bacteria and fungi produce extracellular enzymes to make the substrate in to monomer and available for them. The extracellular enzyme break down the component of historic material and cause deterioration, and/or sometimes the metabolic waste of some microbes can be organic or inorganic acid which also causes biocorrosion of stone monument and buildings (Dakal and Cameotra, 2012).

There are a number of factors for microbial deterioration of historical heritages such as climatic and environmental factors, inorganic and organic pollutant, and component of historic materials. Climatic and environmental factors include sun, humidity, temperature, and rain, the ideal climatic and environmental condition favors the growth of microorganisms. Inorganic and organic pollutant can be factors for deterioration of historical heritages, increasing industrialization and combustion of the fossil fuel has increased the concentration and deposition of organic and inorganic matter on the surface of historic buildings and latter serve as substrate for microbial growth (Gross *et al*., 2006). The composition of some historic material like wood and paint ingredients are made of organic compound like cellulose, hemicelluloses, lignin, and animal fat etc. this organic compound can be substrate and help the succession of microbes on historical heritages (Blanchette, 1991; Iva *et al*., 2013).

Many of known world historic heritage sites affected through microbial induced deterioration, Such as Aztec Ruins and Chaco Canyon great house of New Mexico (Tennessen *et al*., 2002), Magura cave of Bulgaria (Iva *et al*., 2013), Lascaux cave of France (Bastian and Alabouvette, 2009), Kabra-Pahad of India (Jayant *et al*., 2013), and Angkor buildings of Cambodia (Kusumi, 2011).

Ethiopia has a lot of historical heritages which is registered as world heritage by UNESCO and others which requires recognition. These historical heritages provide economic benefit and support the development of the country (www.tourismethiopia.gov.et/ Accessed on July 10, 2014). To get sustainable benefit from our historical heritages periodical assessment and use of different conservative methods has to be taken. But, recent information of the country that shows assessment of microbialy induced deterioration scarce and its control method were not studied. So, the aim of this study is to assess microbial induced deterioration of historical heritages and use of botanicals as a means of management option.

2. Review of literature

2.1. Microbial deterioration

Microbial deterioration is any undesirable changes in a material, caused by microorganisms. There are a number of factors like chemical, biological and physical for deterioration of materials, microorganisms are one of the biological agents for the deterioration of materials. It is geophysical and geochemical process that causes undesirable physical, chemical, mechanical and aesthetic alterations on materials. Many materials like food, drinks, cloth, paper, wood historic monuments and so on are commonly deteriorated by microorganisms. It is a complex process that illustrates the interaction of microorganisms with its substratum and environment. The component of materials and presence of different environmental factors allow for alteration of materials caused by microorganisms (Dakal, 2011).

2.2. Microbial deterioration of wood

Wood is one of the building materials of historical heritages and composed of organic compound like cellulose, hemicellulose, and lignin. The composition of this organic matter is differing from plant to plant. Microorganisms colonize wood and cause deterioration. Over long periods, microbes that are able to grow in adverse condition degrade the wood (Blanchette, 1991). There are a number of factors for deterioration of wood such as moisture, temperature, pH., nitrogen and other nutrients (Eriksson *et al*., 1990). The presence of this all favorable condition provide conducive environment and allow rapid colonization of microbes. The type of wood and presence of extractives within the wood cell also influence decay. Wood from cedar, juniper, cypress, red wood, and oak are more resistant to microbial decay than are pin, birch, beech, aspen, and other wood that have fewer extractives (Zabel and Morrell, 1992).

Some microorganisms are very efficient degraders of cellulose while others degrade extensive amounts of lignin without significant cellulose degradation (Blanchette, 1991).When microorganisms decay wood, they distinct morphological and chemical changes that are signature of the causal organism. Examination of the pattern of microbial attack can reveal what

type of organism was involved and provide a great deal of information on the current condition of the wood (Blanchette, 1991).

Bacteria and fungi are responsible for deterioration of wood. Fungi that cause wood decay classified in to broad categories white, brown, and soft root based on the color and texture of the residual wood after decay (Blanchette, 1998). Bacteria are commonly found in wood and may be associated with decay of fungi or act as scavengers to utilize previously decomposed substrates. They may also be primarily degrader of wood in wet environment. Some bacteria restricted in their capacity to degrade only the pit membrane of wood cell while other type can directly attack the cell wall (Singh and Butcher, 1991).

In New Mexico Soft-rot fungi caused the major type of decay found in wood from historical Aztec Ruins and Chaco Canyon great houses which is the famous historic heritage built approximately 1000 years ago using enormous amount of wood from a variety of tree species (Tennessen *et al*., 2002).

2.3. Microbial deterioration of paints

Paintings are the most valuable part of our ancient cultural heritage, as it has witnessed the presence of prehistoric civilization, their respective sense of creativity and artistic abilities (Jayant *et al*., 2013). They represent different religious events and feasts in separate groups. Paint is essentially the mixture of 1) binder, which adheres the paint to the surface, 2) pigments, which give the paints a color, opacity and occasionally prevent corrosion, and 3) solvents to make the paint spreadable (Ravicumar *et al*., 2012).

The painted surfaces also contain nutrients such as latex, cellulose and the organic compounds (Iva *et al*., 2013) which serve as substrate and later degraded by microorganisms. Paintings are composed of a support (canvas, wood, paper or parchment) a preparation layer and a paint layer, the chemical composition varies according to the mode of painting, the kind of paints used (oil paints, distemper or water color). In stone and building paintings, the preparation is usually made with lime or gypsum with addition of animal or vegetable glue. On this smooth surface several layer of color are present, which consist of pigments mixed with binder of oil or distemper (egg

or glue). The surface is usually covered with a thin, translucent protective varnish (Ravikumar *et al*., 2012).

In painted work of art, the biodeterioration processes can involve either a portion of the painting or all its components. Thus paintings may show trace of biological attack on the reverse side, the support, or on the painted side, and a part or all components may be damage. The organic components in paintings represent a good source of nutrition for wide range of heterotrophic microorganisms. But, biological attacks occur only when there are favorable environmental conditions, and such conditions are often found in museum rooms, old churches or in deposits without any control of the humidity and temperature (Dhawan and Agrawal, 1986).

Paints and coating are susceptible to bacterial and fungal growth when in the liquid state but prone to colonization, especially by fungi, algae and cayanobacteria after application; components such as residual thickening agents are the most abundant carbon source (Gaylard, 2005). The development of micro-fungi on the surface of painting induces aesthetical, mechanical and biochemical decay. In fact, the growing mycelium spread over the paints, masking design and color, while the growth of hyphae and fruiting bodies inside the support can cause friability and loss of the paint layer (Ravikumar *et al*., 2012).

In Bulgaria Magura cave, culturable heterotrophic bacteria were isolated under aerobic conditions from the Gallery with pre-historical drawings. Magura Cave was placed on the tentative list for consideration as a World Heritage Site by UNESCO in 1984. The cave has been opened for visitors only for three years; however, this resulted in changes in cave's micro-climate due mainly to the presence of artificial light and emitted heat, and in enhanced deterioration of many of the drawings by vandal effects. That is why; in 2008 the so called Gallery with the drawing was closed to the public as a conservative measure to preserve the valuable drawings (Iva *et al*., 2013).

In France, Lascaux Cave contained an impressive display of prehistoric art: the main cavern and several galleries connected to it were decorated with engraved, drawn, and painted figures of animals. The approximately 600 paintings, done with mineral pigments mixed with animal fat in

various shades of yellow, red, brown, and black, were dated to the late 15,000 to 13,000 B.C. In 1948 Lascaux Cave was opened to visitors, but in 1963 it was closed indefinitely to the public. Closing was imposed after the discovery of a green patina (from which comes the term *maladie verte*, or green disease) covering the painted portions (Lefe'vre, 1974). Quite unexpectedly, although other algae together with cyanobacteria, bacteria, and fungi were isolated in different parts of the cave, the green patina was composed exclusively of the unicellular alga *Bracteacoccus minor* (order *Chlorococcales*) (Bastian and Alabouvette, 2009).

In India, kabra-pahad is one of the historic places which contain rock paintings. The rock paintings are indeed a representation of fine arts; practiced by early man to decorate their ambient vicinity of shelters. Due to high deterioration it is on the way of losing its importance (Jayant *et al*., 2013).

2.4. Microbial deterioration of stone and buildings

Concrete is one of the strongest construction materials applied in centuries all over the world. During historic time, people used different stones (limestone, granites, marbles etc.) for the construction of magnificent monuments and for making beautiful artworks. These historic building and artworks are our heritage which tells us about the past art, architecture and enriches us with cultural values. Stones used in making these sculptural monuments were highly consolidated and durable and were obtained from naturally occurring sedimentary rocks which are composed of one or more minerals (Takim and Swarajit, 2012).

Microorganisms can cause various damages on the stone surface, such as: formation of biofilm, chemical reactions with substrate, physical penetration into the substrate as well as pigments production (Milica and Jelena, 2009). Biofilm formation on clean surfaces usually starts with phototrophic organisms (algae, cyanobacteria) which use CO2 from the atmosphere as their carbon source and sunlight as their energy source. Heterotrophic organisms (most bacteria and all fungi) need some organic source for their growth, which is provided by metabolites of phototrophic organisms or by air-borne deposition. It has been shown that very low nutrient requirements of some rock inhabiting heterotrophic microorganisms may be fulfilled by remains of polluted air and rain or animal remains and secretion (Suihko *et al*., 2007).

In Cambodia, Angkor is historic building which face deterioration. A very recent study identified the involvement of sulfur oxidizing microorganisms in the deterioration of sandstone. Sulfur-oxidizing bacteria that excrete sulfuric acid are the dominant group of microorganisms involved in exfoliates weathering of sandstone at the Angkor site (Kusumi, 2011).

Fungi are known to harm the monumental stones and artworks in numerous ways. Their growth on stone surface can alter it severely by the excreting inorganic and organic acids as a result of their own metabolism. These metabolically generated organic acids (like oxalic acid and citric acid) have chelating properties by which it weaken the metal-oxygen bond, increases the solubility of some metals and forms complexes with the mineral actions present on the surface matrix (Harely and Gilkes, 2000).

2.5. Microorganism’s impact on stone

The growth and activity of the microorganisms on monuments or stone surface results in five major alterations: bio-weathering (stone dissolution), staining or color alteration, surface alterations (pitting, etching, stratification etc.), biocorrosion and transformation of crystal into small size one (Kusumi, 2011).

2.5.1. Biocorrosion

Biocorrosion of stone can occur through the action of nitrous and nitric acid excreted by nitrifying bacteria (*Nitrosamines* spp. and *Nitrobacter* spp.), leading to stone dissolution and formation of nitrate salts as well as through sulfuric acid excreted by sulfur-oxidizing bacteria (*Thiobacillus* spp.). The acid may react with given constituents of the stone to yield sulfate-based crusts. These sulfates can be precipitated within the pores of the stone and, upon recrystallization, exert considerable stress in the pore walls. Biocorrosion can also occur through the excretion of organic acids by chemoorganothrophic microorganisms, including fungi and lichens. Those acids can also chelate metal actions (e.g., Al, Ca, Fe, Mg, Mn, Si) from minerals to form stable complexes (Warscheid and Braams, 2000; McNamara and Mitchell, 2005). Several chemoorganotrophic bacteria and fungi (*Acidithiobacillus ferrooxidans*, *Bacillus* spp., *Leptospirillum* spp., *Aureobasidium* spp.) are also capable of removing cations, in particular, iron and manganese cationsfrom the mineral lattice by oxidation, contributing to stone deterioration (Warscheid and Braams, 2000; Rawlings, 2005).

2.5.2. Biofilm formation

Algae, microalgae and cyanobacteria are considered as the pioneering inhabitants of a stone surface hence their presence can be easily identifiable on the stones surface (Ortega *et al*., 1991; Crispim *et al*., 2003). Cyanobacteria are often present in association with red algae, green algae and lichens (Crispim *et al*., 2003). These are the one on the major threats to the monumental and ornamental stone works of art. Due to their phototrophic nature, they easily grow on the stone forming colored patinas and incrustations (Tomaselli *et al*., 2000). Their association with substrate in the presence of water makes their growth predominates over other organisms and accelerates the formation of biofilms which facilitates attachment and serves as a mechanism for resisting adverse a biotic condition (Cuzman *et al*., 2010; Gorbushina and Broughton, 2009).

Biofilm act as precursor for the physical damage of stone, it lead to biodeterioration (Ramirez *et al*., 2010; Crispim *et al*., 2003), and discoloration (Hernandez *et al*., 2003). It is believed that under certain conditions almost all substrate both natural and man-made can be colonized by

microorganisms enclosed within a three dimensional extracellular polysaccharides matrices called biofilm (Warscheid and Braams, 2000).

Biofilm composition and distribution mainly depend up on the resulting spatial and temporal variation in a number of abiotic and physico-chemical factors, including micro-environment. Biofilm production on outdoor monuments that are continuously exposed to light tends to contain pleothora of phototrophic microorganisms (Ramirez *et al*., 2010). Biofilm formation represents a mechanism to resist changes in environment like extremes of temperature, drought and prolonged exposure to light, as well as storage of organic carbon and nutrients (Novelo and Ramirez, 2006; Gorbushina and Krumbein, 2000).

2.5.3. Physical penetration

Besides growing on stone surface, microorganisms also colonize the interior of the stone. Fungi can cause serious degradation by physical penetration. Fungal hyphae are able to penetrate deeply beneath the stone surface, contributing to mechanical deterioration. This penetration simultaneously allows the transport of water and nutrients through the stone, which facilitates the colonization of the interior of the stone by bacteria and concomitantly triggers biochemical deterioration (Gómez and de' La, 1994).

2.6. Factors for microbial deterioration of historical heritages

2.6.1. Climatic and environmental factors

Environmental factors have its own impact on the deterioration of historical heritages. Environmental conditions like relative humidity, temperature, wind, light and rainfall plays a crucial role in colonization and establishment of microbial communities on the stone surfaces of monuments and artworks (Dakal and Cameotra, 2011; Ortega *et al*., 1995). The problem is more pronounced in tropical areas where the high temperature, high relative humidity and high annual rainfall favor the growth of diverse group of microorganisms. Microbial growth and activity is a function of the environment that surrounds them. For instance, seepage of the rain water and subsequent dampening and moistening of the vertical walls of the monuments favor the colonization of diverse groups of organisms such as cyanobacteria, algae, fungi and lichens, which foster deterioration (Nuhoglu *et al*., 2006).

2.6.2. Composition of the materials

Raw materials which were used to build historical heritages are mostly organic compounds. Wood is composed of cellulose, hemicellulos, and lignin which serve as substrate for microbial growth. Paintings mainly made from organic binders, pigment, and solvent these organic components represent a good source of nutrition for wide range of heterotrophic microorganisms (Ravikumar *et al*., 2012). The composition of materials can influence the deterioration activity of microorganisms, the type of wood and presence of extractives within the wood cell also influence decay. Wood from cedar, juniper, cypress, red wood, and oak are more resistant to microbial decay than are pin, birch, beech, aspen, and other wood that have fewer extractives (Zabel and Morrell, 1992).

2.6.3. Inorganic and organic pollutant

Industrial and urban activities have modified the composition of the atmosphere, resulting in a more aggressive environment, accelerating the decay of materials. Organic pollutants, carbonaceous particles, dust, pollen, etc. are accumulated at the surface of buildings and trapped in the mineral matrix. This results in the formation of a hard, grey to black crust (Saiz, 1993). Obviously, this crust enriches the Substratum and anthropogenic compounds may influence, to a great extent, the colonization and growth pattern of microorganisms in stones located in polluted environments when compared with the growth of microorganisms in similar stones placed in rural environments (Saiz, 1997).

Increasing industrialization and combustion of the fossil fuel has increased the concentration of SO2 and NO2 in the atmosphere. Both NO2 and SO2 have bioweathering effect. SO2 together with other dark particles (particulate matters) settles down and get deposited on the stone surfaces rendering darkened and yellowish color to them (Gross *et al*., 2006). There is a special class of bacteria, called sulfur-oxidizing bacteria and nitrifying bacteria (chemoautotrophs) which can colonize marble surface and oxidize the nitrogen compounds including atmospheric ammonia (*Nitrosomonas* spp. and *Nitrobacter* spp.) and sulfur compounds (*Thiobacillus* spp.) into nitric and sulfuric acid respectively. These acids are highly corrosive and accelerate the dissolution of the stone surface (biocorrosion) and changes (Dakal and Cameotra, 2012).

2.6.4. Humans' as a factor contribution for historical heritages deterioration

Human beings are closely related with historical heritages, peoples reveal their belief, culture and attitude with their architectural and aesthetical skill. Many microorganisms inhabit human skin as normal flora or resident flora, human contact with historical heritages allow for inoculation of microorganisms. Many bacteria are found on human skin among them *Staphylococcus* spp. is the common normal human flora, typically the skin flora, and less commonly the mucosal flora (Bek, 2008).

Mainly religious historical heritages still give service and peoples made contact with their hands, forehead and other body parts to get mercy and blessed by God. As a result there will be

incidence to inoculate microorganisms and colonization of microbes later causes historical deterioration. There is a condition that historical heritages are deteriorated as a result of exposition to human being, the Magura cave of Bulgaria closed due to such kind of problem (Iva *et al*., 2013).

2.7. Microorganisms associated with deterioration of historical heritages

Microorganisms involve in the deterioration of historical heritages. The most common microbes which frequently occurred are bacteria and fungi. Fungi and bacteria found on mural paintings in churches, caves and catacombs, and even as biodeteriogens of architectural surfaces and stone monuments in outdoor environments (Ettenauer *et al*., 2010; Piñar and Sterflinger, 2009; Saarela *et al*., 2004; Steiger *et al*., 2010; Sterflinger, 2000; Urzì, 2004).

2.7.1. Bacteria

Many species of bacteria promote deterioration of historical heritages. They produce extracellular enzyme to change the substrate in to monomer form in order to make it available. The substrate may be the part of the heritage as a result due to enzymatic activity it will deteriorate. And sometimes the metabolic waste of some bacteria is organic acid, this cause deterioration of stone/concrete, paintings, and wood material. Bacteria involved in deterioration of monuments and artworks mainly belong to three nutritional groups: Photoautotrophs, Chemolithoautotrophs and Chemoorganotrophs. Among phototrophs and chemolithoautotrophs are mainly cyanobacteria, sulfur-oxidizing and nitrifying bacteria were reported from the heritage sites. Due to their simpler nutritional (like inorganic minerals, atmospheric ammonia etc.) and ecological needs (like presence of light, CO_2 and water) these bacteria easily develop on outdoor monuments (Dakal and Cameotra, 2012).

Cyanobacteria are usually present in association with green and red algae and diatoms. The dominance of one or another of these groups varies both locally and regionally. Cyanobacteria and green or red algae growing on surfaces within buildings (e.g., churches or grottos) are well

Adapted to survive at very low light levels. Cyanobacteria and algae can form biofilms and crust on rock surfaces that are deep or bright green under humid conditions and deep black when dry. Apart from the evident aesthetic damage on the surfaces, there is much evidence of significant Physical and chemical deterioration of the material by excretion of chelating organic acids and Sugar-derived carbonic acids, which initiate the perforating activity (Urzi *et al*., 2004).

Bacteria are also involved in biofilm formation followed by reduced durability of structure. While fungal and algal growths are often visible to the naked eye, bacteria can be present no an apparently clean surface in sufficient numbers to exert adverse effect. Such effect can include concrete corrosion due to production of inorganic acids (Cragnolino and Tuovinen, 1984; Bock and Sand 1986; May *et al*., 1993), or blistering of paint due to other metabolic activities (Johannessen and Norgaard, 1991). Chemolithotrophic and oligotrohhic bacteria, which grow at very low nutrient levels, may condition a surface, making it more amenable to colonization by other microorganisms (May *et al*., 1993).

There are a number of bacteria which involved in deterioration of different material of historical heritages. Some of the bacteria which identified on different material of historical heritage listed below on the table.

Table.1. Biodeteriorating bacteria on different materials.

Material	Bacterial group
Concrete, stone and mortars	Sulfur-oxidising bacteria (e.g. *Thiobacillus*),heterotrophicbacteria(e.g. *Arthrobacter,Micrococcus, Pseudomonas), nitrifying bacteria*
Painted Surfaces	*Micrococcus luteus, Serratina spp., Staphylococcus, Flavobacterium,Pseudomonas fragi. P. fluorescens*

Adopted from: Ravikumar *et* al., 2012.

2.7.2. Fungi

Fungi are among the most harmful organisms associated to biodeterioration of organic and inorganic materials. It is well known, in fact, the metabolic versatility of this group of microorganisms that enhance their efficiency to colonize a very different kind of substrata (wood, glass, stone, and book). In the last decade more evidences have been shown that in rock material, the most common fungal colonizer is the so-called black meristematic fungi. They are commonly isolated from the sun exposed surfaces in Mediterranean as well as from dry and cold climates (Anagnostitidis *et al*., 1991; Urzì *et al*., 2000a). Their occurrence on the stones is reported to be combined not only with aesthetical spoiling of the monuments, due to color changes and black spots, but also with a strong evidence that these organisms are causing crater shaped lesions, chipping and exfoliation of the rock surfaces combined with the loss of materials (Urzì *et al*., 2000b).

The fungi secrete extracellular enzymes which break down potential food source, and absorb nutrients back in to the fungal colony. They damage standing timber, finished wood products, fibers, and a wide range of non-cellulosic product such as plastics, fuels, paints, glues, drugs, and other human artifacts (Onions *et al*., 1981; Rose, 1981). For instance, in fungal colonization of mural paintings (Saiz and Samson, 1981) have shown that, at the beginning, growth of fungi on a mural's surface caused only aesthetic damage since there was little or no alteration of the painted surface. Later on, fungal growth in depth occurred. Hyphae penetrated the painted layer, degrading some of its components (especially glues and binders), which resulted in a decrease in the cohesion of the painted layers, thus giving rise to exfoliations, cracking, and loss of the paint. To these damages one should add those inflicted by metabolites, often acidic in nature, and by extracellular enzymes excreted by microorganisms. These compounds may modify the colors as well as the stability of the painted layer and of the substrate (Ravikumar *et al*., 2012).

The production of acids by several filamentous fungi isolated from stone buildings in Brazil has been reported and present evidence for the acid attack of the fungus *Aspergillus glaucus* on concrete (Resende *et al*., 1996; McCormack *et al*., 1996). There are a number of fungal species

which deteriorate different material of historical heritages. Some of them which are identified on different material listed below on table 2.

Table.2. Biodeteriorating fungi on different materials.

Material	Fungal species
Painted surface	*Alternaria, Aspergillus, Aureobasidium,Cephalosporium; Cladosporium, Curvularia, Exophiala, Fusarium, Geomyces, Mucor; Penicillium, Stachybotrys, Stemphyllium, Trametes, Trichoderma,Ulocladium, Verticillium*
Wood	Brown- rot fungi, white- rot fungi,
Concrete and stone	*Alternaria, Aspergillus, Aureobasidium, Botrytis, Candida, Cladosporium, Curvularia, Exophiala, Fusarium, Mucor, Paecilomyces, Penicillium, Phoma, Sporobolomyces, Trichoderma, Verticillium*

Adopted from: Ravikumar *et* al., 2012.

2.7. Antimicrobial effect of botanicals

Medicinal plant represents a rich source of anti-microbial agents (Mahes and Satish, 2008). Due to the anti-microbial effect of some plant extract it may involve in disinfection of microbial deterioration of historical heritages. The botanical materials include crude extracts, essential oils and isolated or purified compounds from various plants species and commercial products. Plants contain a wide spectrum of secondary metabolites such as phenols, flavonoids, quinones, tannins, essential oils, alkaloids, saponins and sterols. Such plant-derived chemicals may be exploited for their different biological property; which constitutes an important source of microbicides, pesticides and many pharmaceutical drugs. Numerous studies have documented about antifungal and antibacterial effect of plant essential oils (Canillac and Mourey, 2001).

2.8. Current attention to prevent microbial deterioration

At present, most of the well-known and visited caves containing valuable pre-historical paintings are affected by progressive microbial colonization and biodeterioration, and the control, treatment, and preservation of these specific environments is a serious worldwide problem (Cañaveras *et al*., 2001; Allemand and Bahn, 2005; Fox, 2008).

World Heritage Committee (WHC) is intergovernmental organization, which is responsible for cataloging and protecting World Heritage sites, operates under the direction of the United Nations Educational, Scientific and Cultural Organization (UNESCO). It was established to implement the terms of the World Heritage Convention, an agreement adopted by the General Conference of UNESCO in 1972. World heritage committee gives support for different country to take action on the conservation and restoration of historical heritages. The success story of WHC indicates it was possible to restore heritages like Angkor, Cambodia and The Old City of Dubrovnik in Croatia with the collaboration of state government (www.swissinfo.ch/eng/unesco-world-heritage-a success story/ Accessed on July 3, 2014).

Many countries like Italy privilege institutions to follow and treat microbial induced deterioration of historical heritages. Cleaning of these damages generally requires chemical and/or mechanical methods, these methods are not always entirely satisfactory and the institutions develop project to conserve their historical heritages from deterioration (www.savingantiquities.org/--Italy/ Accessed on June 20, 2014).

2.9. Statement of the problem

At present, most of the well-known and visited historical heritages containing valuable pre-historical paintings are affected by progressive microbial colonization and biodeterioration, and the control, treatment, and preservation of these specific environments is a serious worldwide problem (Cañaveras *et al*., 2001; Allemand and Bahn, 2005; Fox, 2008). Heritage buildings collapsed due to biocorrosion of stone and concrete caused by metabolic wastes of microorganisms (Warscheid and Braams, 2000).

Historical heritages have valuable meaning to the existing peoples, it reveal religious belief and civilization of the ancient peoples with magnificent architectural and aesthetical skill. Loss of these historical heritages is just elimination of tangible historic evidence of pre-existing peoples. Many historic heritages damaged as a result of microbial induced deterioration, such as Aztec Ruins and Chaco Canyon great house of New Mexico (Tennessen *et al*., 2002), Magura cave of Bulgaria (Iva *et al*., 2013), Lascaux cave of France (Bastian and Alabouvette, 2009), Kabra-Pahad of India (Jayant *et al*., 2013), and Angkor buildings of Cambodia (Kusumi, 2011).

Ethiopia is ancient country containing many magnificent historical heritages made by pre historic peoples. Practices to protect and conserve historical heritages is supported by Law, 1958 (EC) 'Antiquities Administration' and Proclamation No. 209/2000, the revised proclamation for Research and Conservation of Cultural Heritage provides the national legislative background for the protection and preservation of the Ethiopian cultural heritage. However, Taking action at governmental level is challenging as a result of inadequate skilled man power, budget, and constitutional character which means the EPDRF government is not in contact with religious institution and Monuments that are used for religious services are under the direct responsibility of the Ethiopian Orthodox Church (www.tourismethiopia.gov.et/Accessed on July 10, 2014).

2.10. Significance of the study

Ethiopia has a lot of Historical heritages which express the ancient civilization of the country. Many of the heritages related with Orthodox Church are still gives service. The presences of these historical heritages provide income and support economic development of the country. Assessment and conservation of microbial induced deterioration of historical heritages is necessary, Loss of historical heritage is not only a matter of economic crisis but also cause historical crisis. So, the result of this study was investigated microbial induced deterioration of historical heritages and determines antimicrobial effect of botanicals as management option.

3. Objective

3.1. General objective

- The main objective of this study was to investigate microbial induced deterioration and use of botanicals as a means of management options, on historical heritages of Gondar town.

3.2. Specific objective

- To isolate, characterize and identify the microorganisms commonly found on historical heritages.
- To screen biodeteriorating microorganisms on historical heritages.
- To evaluate the antimicrobial effect of botanicals on common isolates as a management options.

4. Material and methods

4.1. Study area

Gondar is one of the historic places, which is located North West Ethiopia mainly in Amhara regional state, 748k.m away north of Addis Ababa. It is 2260 meter above sea level and bounded by 12^0 36' north and 37^0 28' east longitude. The annual rainfall is1172mm and main relative humidity for an average is recorded as 55.7% and on monthly basis it range from 40% in January and February to79% in July. The study area includes three most frequently visited and well known historical heritages named Fasil castle (world heritage), Qwesquam church, and Debre Berhan Selassie church (www.tourismethiopia.gov.et/ historical heritages conservation. Accessed on July 10, 2014). The study was conducted from July 2014-December, 2014.

4.2. Study design

The study design was conducted by using experimental based type. Evaluation of microbial induced deteriroration on historical heritages of Gondar town and use of botanicals as a means of management option were done.

4.3. Sampling technique

The sampling technique was purposeful (non probable method), samples were collected only on the deteriorated surface of historic heritages.

5.4. Sample size

A total of 60 samples were collected from painted surface, stone/concrete, and wood surface of Fasil castle (world heritage), Qwesquam church, and Debre Berhan Selassie church.

4.5. Sample collection and preparation

A total of 60 samples were collected from deteriorated and undeteriorated wood, wall paintings, and stone/concrete surface of well known historical heritages of Gondar town named Fasil castle

(world heritage), Qwesquam church, and Debre Berhan Selase church from July 2014 to august 2014. By using sterile cotton swab and moisten with distilled water vigorously rubbed the surface and swab samples were immersed and put in to ice box and carefully transported to Gondar university microbiology laboratory for examination. Swab samples diluted in 10 ml sterile distilled water and shaken mechanically using vortexer. Then serial dilution done with peptone water for bacteria and distilled water for fungi.

4.6. Characterization and identification of bacteria

The pure cultures of bacteria isolated from the various samples were prepared and bacterial colonies identified based on morphological identity and biochemical test. Bergey's Manual of Systematic Bacteriology (Schleifer, 1989; Aneja, 2005) and UK standard for microbiology investigation (Barrow and Feltham, 1993) were a guide line for identification.

4.7. Identification of fungi

For isolation and identification of fungi, Potato Dextrose Agar (PDA) and cezapack dox agar media were prepared containing chloramphenicol (500 mg/liter) to control the bacterial growth in the same. 0.1 ml washed samples were poured in to PDA and the plates were incubated at 30 ± 1^0c and examined daily for the growth rates and sporulations, continuously for 7 days. After completion of incubation period, the various isolated fungal colonies were transferred into fresh PDA and cezapack dox agar plates the procedure was repeated 3 times to remove impurities. Macroscopic and microscopic identification were done using guide lines.

4.8. Plant leaves collection

The plant material that used in this work were freshly harvested leaves of *Azadirachta indica* (Neem), *Datura Stramonium* (Atsefaris), and *Ricinus communis* (Gulo). Collection of *Datura Stramonium* (Atsefaris) and *Ricinus communis* (Gulo) were done around Gondar town while *Azadirachta indica* (Neem) was from Metema northwest Ethiopia in the month of July 2014.

Plant species were identified by verifying the color pictures followed by description and identification characters (Bekele, 2007).

4.9. Plant leaves extraction

The plant leaves were thoroughly washed with tap water followed by distilled water to avoid dusts and other unwanted materials accumulated on the leaves. The dust free leaves were dried in an oven at 50°C for two days. The dried leaves were powdered by using electric blender and fine powder collected from the powdered leaves through sieving. Powdered plant leaves were macerated in different solvent like ethanol (100%), acetone/chloroform (1:1 ratio), and water with 10 g to 100 ml ratio and shacked well on mechanical shaker for 24hr. The mixture were kept undisturbed at room temperature for 24 hrs in a sterile flask covered with aluminum foil to avoid evaporation and subjected to filtration through sterilized cheese cloth followed by Whatman no.1 filter paper. After filtration, the extract was evaporated in Rota vapor and semi solid substance was finally dried in an oven and the powder crude extract will keep at 4^0C for further use (Barreto *et al.*, 2002).

4.10. Antimicrobial assay

4.10.1. Disk diffusion assay

The disk diffusion test was performed using the standard procedure of National Committee for Clinical Laboratory Standards (NCCLS) (www.worldcat.org/identities/nc- the national committee for clinical laboratory standards/Accessed on June 15, 2014). The inoculums suspensions of each bacterial and fungal isolates were swabbed on the entire surface of Mueller-Hinton agar and PDA respectively. Sterile 6-mm filter paper discs were macerinated on the different concentration of crude extracts and aseptically placed on using sterile forceps to pick the paper discs and placed on the inoculated plates. The plates were left at ambient temperature for 15 min to allow excess prediffusion of extracts prior to incubation. Finally after the incubation period finished the diameter of inhibition zones were measured.

4.10.2. Determination of minimum inhibitory concentration

The minimum inhibitory concentration is the concentration giving the least inhibitory activity and below which there is no further inhibition. It is therefore regarded as the concentration giving the lowest possible zones of inhibition. Two fold serial dilutions were prepared to obtain a concentration range (100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml). Sterile discs were immersed into the different dilutions and absorption was allowed for 30 minutes. The disks aseptically placed on and diameters of inhibition zones were measured after incubation at right time.

4.10.3. Screening of phytochemicals

Plant phytochemicals were screened using deferent indicator chemicals. Few drops of ferric chloride solution were added in filtrate. A greenish black precipitate indicates the presence of tannins. Chloroform and concentrated H_2SO_4 was added in filtrate a reddish brown color or ring indicates the presence of terpenoids. Filtrate was dissolved in dilute HCL and saturated picric acid was added. A yellow precipitate indicates the presence of alkaloids. Few drops of lead acetate were added to the filtrate a yellow precipitate indicates the presence of flavonoids. Concentrated H_2SO_4 was added in filtrate red coloration indicates the presence of steroids.

4.11. Data analysis

The data collated from all the experiments were subjected to the analysis of variance (ANOVA) and appropriate statistical analysis system (SAS) and /or SPSS computer software 16 version.

5. Result and Discussion

Result

Microbial investigation

A total of seven bacteria and eleven fungi were screened from the three historic place of Gondar town. The historic materials of Qwesquam church contaminated by microbes among from the materials the wall painting of the church highly colonize by bacteria and fungi. *S.epidermidis* and *S.aureus* are mostly occurred on different materials of the church. *A.niger* and *A.fumigates* are also dominantly colonized among fungal species. Relatively the wood part of the church show reduced microbial population than wall paintings and stone/concretes.

Table.**3.** Microorganisms screened from Qwesquam church from July to August 2014, Gondar town

	Material		
Microorganisms	Wood	Wall paintings	Stone/concretes
Bacteria	*P.aeruginosa* *E.coli* *S.epidermidis*	*S.epidermidis* *S.aureus* *Salmonella spp* *Yersinia* *E.coli*	*Kellebsela spp* *S.epidermidis* *S.aureus* *P.aeruginosa*
Fungi	*A.niger* *A.flavus* *P.citrinum* *A.fumigates*	*A.niger* *Trichoderma spp* *A.candidus* *F.solani* *A.fumigates* *A.alternata*	*A.flavus* *Mucor spp* *P.citrinum*

Investigation of microorganisms found on different parts of Debre Berhan Selassie church reveals colonization of microbes. In contrast with Qwesquam church the microbial load of Debre Berhan Selassie church was high. The door and window of the church which are made of wood revealed high contamination of microbes. *P.aeruginosa*, *S.epedermidis* and *E.coli* were commonly found on the wood and wall paintings of the church.

Table.4. Microorganisms screened from Debre Berhan Selassie church from July to August 2014, Gondar town

Microorganisms	Materials		
	Wood	Wall paintings	Stone/concretes
Bacteria	*S.aurues* *S.epedermidis* *P.aeruginosa* *E.coli*	*E.coli* *E.epidermidis* *Salmonella spp* *Kellebsela spp*	*S.aurues* *E.epidermidis* *P.aeruginosa*
Fungi	*A.fumigates* *A.niger* *Trichoderma spp* *P.citrinum* *Mucor spp*	*A.flavus* *A.niger* *F.solani* *A.terrus*	*P.citrinum* *F.solani* *Mucor spp* *A.alternata*

Table.**5.** Microorganisms screened from Fasil castle from July to August 2014,

Microorganisms	Materials	
	Wood	Stone/concretes
Bacteria	*S.aurues* *S.epidermidis*	*E.coli* *P.aeruginosa* *E.epidermidis*
Fungi	*A.niger* *F.solani* *Trichoderma spp* *Mucor spp*	*A.niger* *A.alternata* *P.citrinum* *A.candidus* *C.oxysporum*

Prevalence of microorganisms

The percent prevalence of microorganisms among 60 samples which was taken from different parts of historical heritages reveals that *E.epidermidis* occurred 31% of the samples which was the highest from bacterial population. Among fungal species screened from historical heritages *A.niger* registered 20% occurrence.

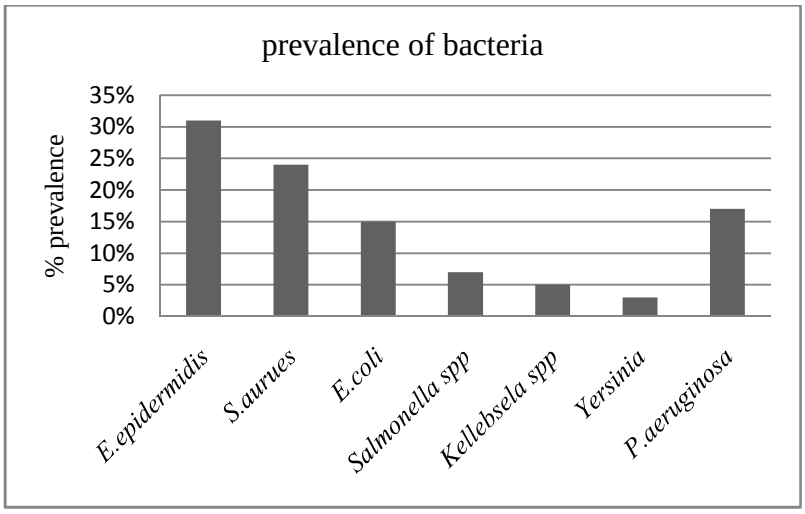

Figure.1. prevalence of bacteria from the total samples.

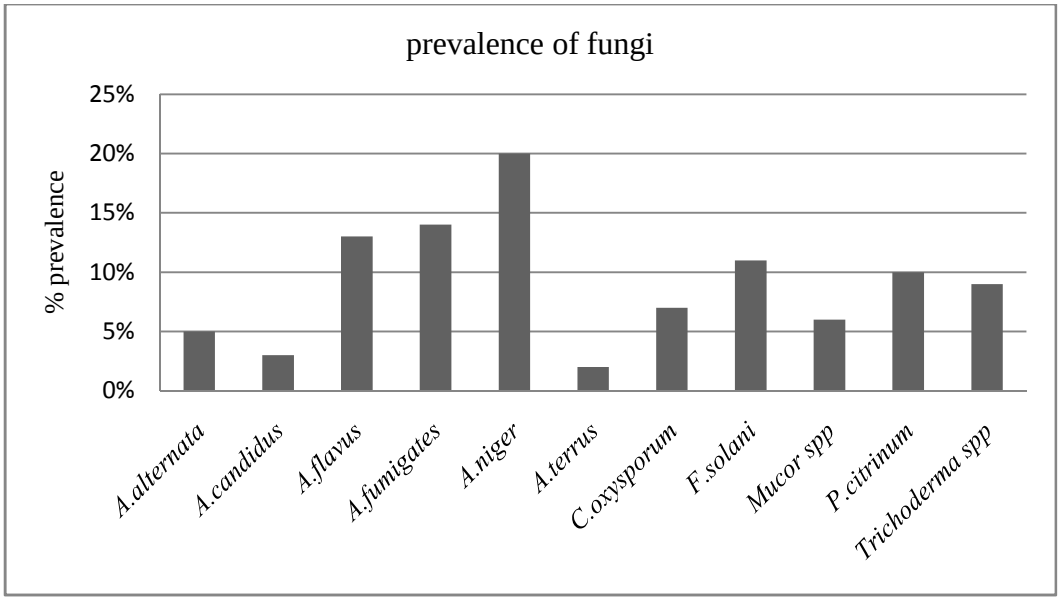

Figure.2. prevalence of fungi from the total sample.

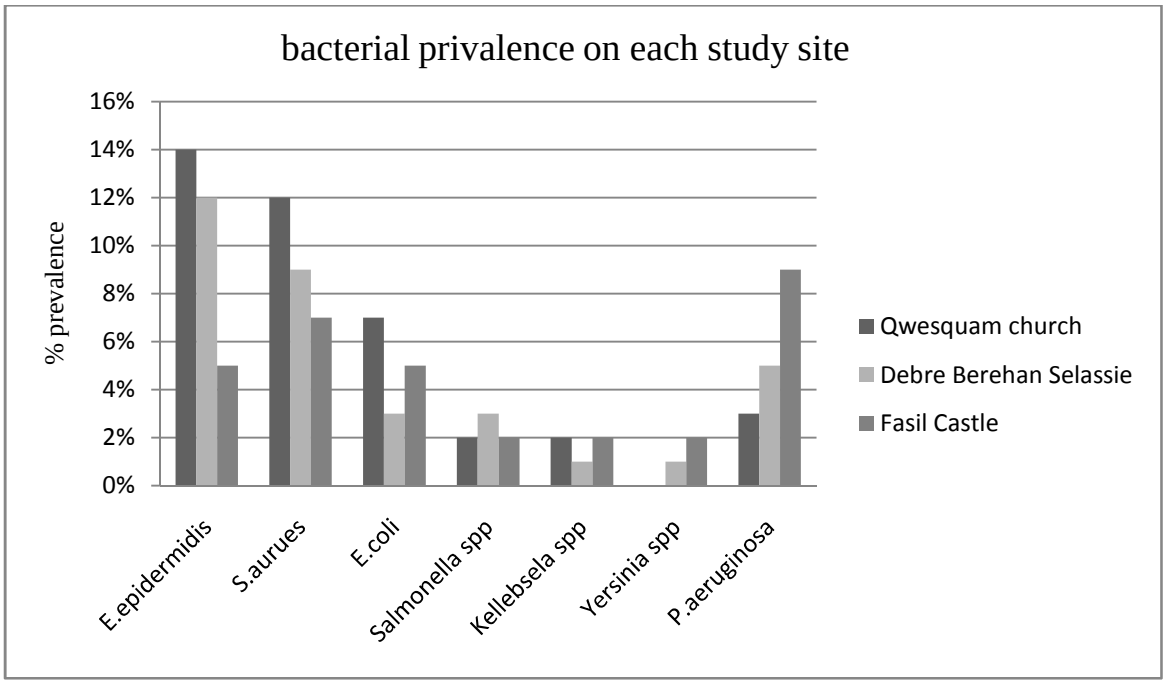

Figure.3. Bacterial prevalence on each study site

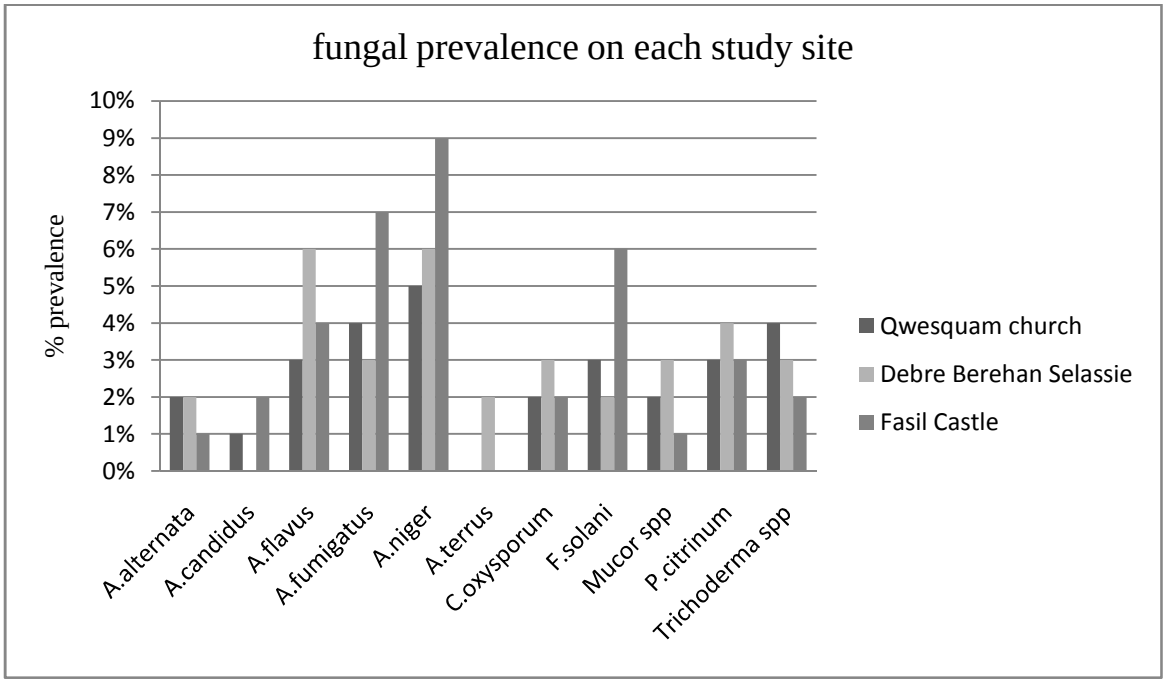

Figure.4. Fungal prevalence on each study site

Antimicrobial effect of botanicals

Test of anti microbial effect of common medicinal plants on selected strain of bacteria and fungi were done. Selection of bacterial and fungal strain is based on the highest colony forming units of organisms which appeared on those historical heritages. Solvents like water, ethanol, and acetone/chloroform were used to prepare the crude extract. *Staphaylococcus epidermidis* show highest sensitivity for acetone/chloroform extract of *Azadricheta Indica* while it resistant for water extract of the three medicinal plants. Vancomycin and penicillin were used as positive and negative control respectively. *Aspergillus Niger* show highest sensitivity for acetone/chloroform extract of *Azadricheta Indica,* alcohol and water were a positive and negative control.

Table.6. Sensitivity test of botanicals against *Staphylococcus epidermidis.*

Strain	plant species	Solvent	Diameter of inhibition zone(mm)				Van	Pen
			100 Mg/ml	50 Mg/ml	25 Mg/ml	12.5 Mg/ml		
		Water	-	-	-	-	S	R
	Datura stramomium	Ethanol	b	c	-	-	S	R
		Acetone/ chloroform	b	c	-	-	S	R
Staphaylococcus Epidermidis	*Azadricheta indica*	Water	-	-	-	-	S	R
		Ethanol	ab	ab	b	-	S	R
		Acetone/ Chloroform	a	a	ab	-	S	R
	Rcinus comminus	Water	-	-	-	-	S	R
		Ethanol	b	c	-	-	S	R
		Acetone/ chloroform	ab	bc	B	-	S	R

Table.7. Sensitivity test of botanicals against *Aspergillus niger*.

Strain	plant species	Solvent	Diameter of inhibition zone(mm) 100 Mg/ml	50 Mg/ml	25 Mg/ml	12.5 Mg/ml	Alcohol	Water
		Water	c	d	-	-	S	R
	Datura stramomium	Ethanol	ab	bc	c	-	S	R
		Acetone/ chloroform	b	bc	b	-	S	R
Aspergillus Niger	*Azadricheta indica*	Water	d	d	-	-	S	R
		Ethanol	a	ab	b		S	R
		Acetone/ Chloroform	a	a	a	-	S	R
	Rcinus comminus	Water	e	-	-	-	S	R
		Ethanol	b	c	b	-	S	R
		Acetone/ Chloroform	ab	b	b	-	S	R

Determination of minimum inhibitory concentration

Determinations of minimum inhibitory concentration (MIC) of botanicals were done by disk diffusion assay. For *S.epidermidis* the minimum inhibitory concentration 25 mg/ml result on both ethanol and acetone/chloroform extract of *Azadricheta indica* and the same concentration of acetone/chloroform extract of *Rcinus comminus.* The minimum inhibitory concentration of botanicals against *A.niger* recorded 25 mg/ml for ethanol and acetone/chloroform extract of the three medicinal plants.

Table.8. Minimum inhibitory concentration of botanicals

Strain	Plant Species	Plant extract mg/ml Water	Ethanol	Acetone/chloroform
S.epidermidis	*Datura stramomium*	-	50	50
	Azadricheta indica	-	25	25
	Rcinus Comminus	-	50	25
A.niger	*Datura stramomium*	50	25	25
	Azadricheta indica	50	25	25
	Rcinus Comminus	50	25	25

Screening of phytochemicals

Screening of Phytochemical on ethanol and acetone/chloroform extract of *Datura stramonium*, *Azadirachta indica*, and *Ricinus commnuis* were done by identifying the appeared color change after the addition of indicator chemicals.

Table.9. Screened phytochemicals

phytochemicals	Plant					
	Datura stramonium		*Azadirachta indica*		*Ricinus commnuis*	
	ethanol	Aceto/chlor	ethanol	Aceto/chlor	ethanol	Aceto/chlo
Flavonoids	+/+		+/+		+/+	
Alkaloid	+/+		+/+		+/+	
Tannins	+/+		+/-		+/-	
Terpenoids	-/+		-/+		-/+	
Steroids	+/+		+/+		+/+	

Discussion

The doors and window of the three historic heritages of Gondar town made from wood mainly from Junipers plant, which contain high lignin component (Zabel and Morrell, 1992). As table 4 indicates that the wood surface of Debre Berhan Selassie church was highly contaminated than the other sites. *S.epidermidis, S.aurues, E.coli,* and *P.aeruginosa* were most commonly occurred bacteria and *A.niger* and *A.fumigates* were dominant fungi on the wood surface of the three historical heritages. Bacteria are commonly found in wood and may be associated with decay of fungi or act as scavengers to utilize previously decomposed substrates (Singh and Butcher, 1991). The screened bacteria found on the wood surface of the historic site of Gondar town pointed that there is human contamination because of they are normal flora of human skin.

Mainly human contact with the doors and window can be the means for inoculation of organisms. *S.epidermidis* and *E.coli* are the dominant one which are known to form biofilm and they associated with decay fungi or act as scavengers to utilize previously decomposed substrates, as a result cavitations appeared on the wood surface (Blahchette, 2003). As tables 3, 4 and 5 indicate that *A.niger* and *A.fumigatus* are the dominant fungal strain screened on wood surface, *A.niger* commonly cause black pigmentation. According to Theodore and Ellis (1966) Mold fungi discolor the wood by developing pigmented spores on the surface; they also may cause shallow discolorations within the wood.

The wall paintings of churches made from organic and inorganic materials which can be serve as substrate for microorganisms. The wall paintings were contaminated by bacteria and fungi among them *E.epidermidis* and *A.niger* microbial the most dominant one. The churches still give service and many followers and visitors made contact with the religious wall paintings this and the composition of paint materials allow for inoculation and succession of microorganisms. Human contact is a means of contamination that is why, in 2008 Magura Cave the so called Gallery with the drawing was closed to the public as a conservative measure to preserve the valuable drawings (Iva *et al*., 2013).

Microbial deterioration of historical wall painting can cause pigmentation, lose of materials and hiding of art works by forming biofilm. *Aspergillus niger*, which dominantly occurred in the sample provide black pigmentation and with some other fungi is already known to produce such enzymes, which were enriched with various organic acids like gluconic, citric, and oxalic acids that can solubilize paint ingredients. As table 3 and 4 indicate that microbial load found on the wall paintings of churches was greater than the wood and stone/concrete surfaces, this is because of the paint contain organic and inorganic constituent and this can be good nutrient source for microbial growth (Jayant *et al.*, 2013).

Investigation of microbial induced deterioration on the stone/concrete of the three historic heritages of Gondar town reveal colonization of microorganisms. Due to the exposition to air organic and inorganic compounds accumulate on the outer surface of wall/concrete and serve as a substrate for microbes which colonize the surface (Saiz, 1993). In the context of Gondar town historical heritages black pigmentation occurred on the surface of monuments. the excretes of birds can be food source for colonizing microorganisms which cause deterioration as result of acid corrosion, enzymatic degradation and mechanical attack (Ahinkafi and Haruna, 2013).

Tables 6 and 7 indicate that the antimicrobial test of those water, ethanol and acetone/chloroform extracts of *Datuta stramonium, Azadichita indica and Ricinus comminus* was investigated using disk diffusion method against *S.epidermidis* and *A.niger* which was recorded the highest prevalence. All the examined extract showed varying degrees of antimicrobial activities against the test organism. The reason why botanicals select as management option was, it is easy to process, eco-friendly, easily bio-degradable, cheaper, and reduce crop losses (Malkhan *et al.*, 2012).

Table 6 indicates the antibacterial activity of the acetone/chloroform extract of *Azadichita indica* showed the maximum inhibition zone against *S.epidermidis*. 25 mg/ml of the ethanol and acetone/chloroform extract of *Azadichita indica* and the same concentration of acetone/chloroform extract of *Ricinus comminus* recorded as the minimum inhibitory concentration against *S.epidermidis.* The presence of bioactive compounds which is extracted by the above solvents enables the plants to inhibit the growth of microorganisms. No inhibition for water extract at all because water soluble plant bioactive compounds has less antimicrobial effect (Das *et al.*,2010).

Table 7 indicate the antifungal assay of *D.stramonium, A.indica and R.comminus* against *A.niger* reveals that the acetone/chloroform and ethanolic extract of *A. indica* have good antifungal effect respectively, and No inhibitions for water extract of *R.comminus.* The minimum inhibitory concentration for inhibition of *A.niger was* 25 mg/ml; the concentration is good enough to apply on materials. Antibacterial activity of *Azadiracta indica* was analyzed by previous workers showed that the chloroform extract of leaves possess significant activity, than petroleum ethar and methanol extracts. Early studies proved ethanol as the most efficient solvent for extracting broad spectrum of antibacterial compounds from plants (Vinoth *et al.,* 2012)

The variation of antimicrobial efficacy of medicinal plants could be the type of solvent that used, plant part used, extraction procedure and the type of bioactive compound found in the plant part. phytochemicals can be extract through different solvent based on the polarity. The screening test of phytochemicals on *D.stramonium, A.indica* and *R.comminus* with ethanolic and acetone/chloroform extract were done to find active chemical constituent. The tests were done for flavonoids, alkaloids, tannins, terpenoids and steroids. Except terpenoids the other phytochemicals extracted through acetone/chloroform. The presence of these phytochemical components may be responsible for the observed antimicrobial activity of the plant leaf extract. Different solvent extract different phytochemicals which is found in the same plant this is why the acetone/chloroform extract is effective than ethanol extract (Malkhan *et al.,* 2012).

There are proclamations stated nationally and internationally to conserve and restore historical heritages. *E.epidermidis* and *A.niger* were highly contaminating the three historical site of Gondar town use of botanicals to treat microbial induced deterioration revealed promising result. *UNESCO* world heritage committee recommends using biological mechanism rather than chemical and physical approach to treat microbial induced deterioration of historical heritages.

Conclusion

The historic heritages of Gondar town show some level of deterioration induced by microorganism. This is because of divers factors like regular body contact of peoples with historic materials which leads to inoculation of microorganisms, the composition of historic materials which can be a substrate for microorganism, and the presence of favorable climatic and environmental condition contribute for the succession of microorganism. The result of this study indicates microbial contamination found on the historical heritages of Gondar town and to treat this microbial deterioration common medicinal plant become effective. The acetone/chloroform extract of *A.indica* reveal promising result against *S.epidermidis* and *A.nige.* For a country like Ethiopia historical heritages provide economic capacity and promote the culture of the peoples, so just to get such benefits we have to conserve our historical heritages protecting them from microbial deterioration and chemical deterioration should not be left for the coming generation.

Recommendation

Recommendation based on this thesis result will be given for tourism bureau and church administrators. Paying attention for the conservation of historical heritages is spring board to carry on well organized conservation and preservation.

Keeping these valuable historical heritages from deterioration caused by biological, chemical and physical factors based on periodical assessment by professionals must be conduct.

Keeping away those from body contact reduces the incidence of microbial colonization on historical heritages.

Use botanicals for management of microbial induced deterioration of historical heritages.

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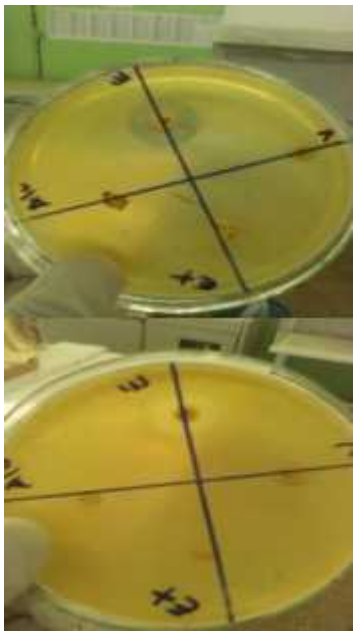
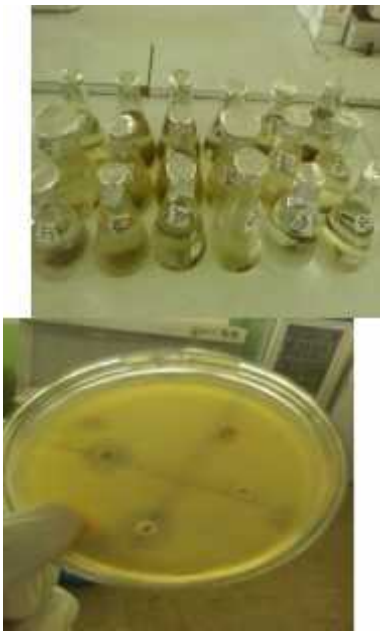
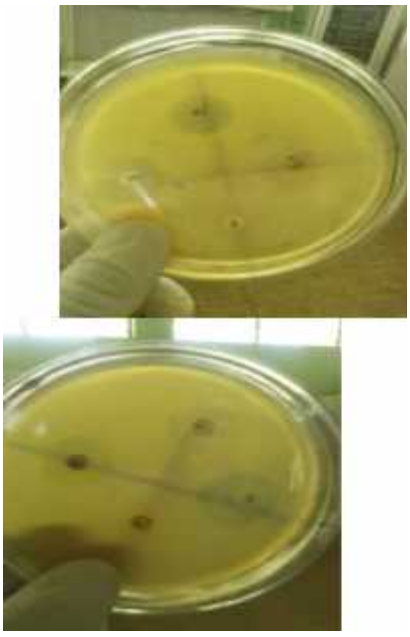
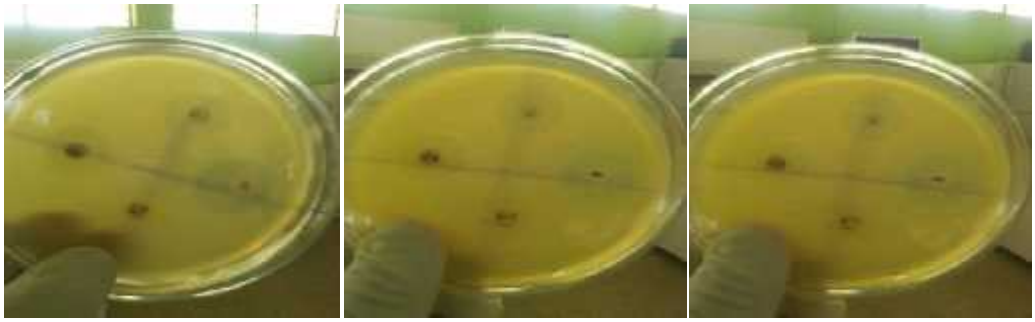
Annex

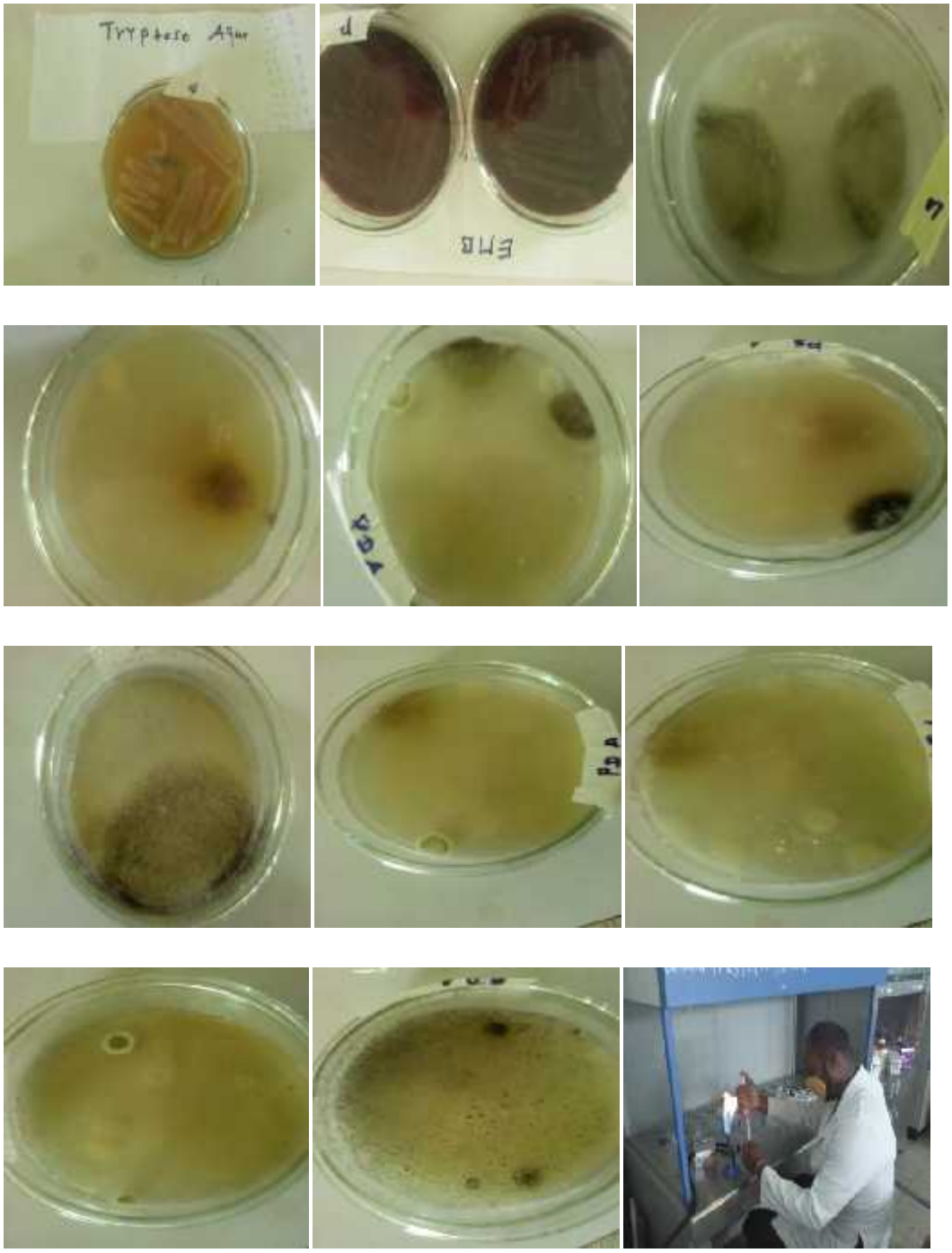
Photo










Tryptase Agar